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Diploschistes wui sp. nov., an overlooked saxicolous lichen from Northwestern China

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Abstract—*Diploschistes wui* is described as a new species, based on three collections from Xinjiang in Northwestern China. It is characterized by a bluish gray and thin thallus, epruinose discs, small, 8-spored asci, and small ascospores. The new lichen resembles *D. rampoddensis* but is readily distinguished by the absence of surface crystals and pruinose discs. It grows on rocks at elevations above 1800 m. Our ITS rDNA sequence analyses support this species as independent.

Key words—biodiversity, central Asia, *Graphidaceae*, *Ostropales*, taxonomy

Introduction

The lichen-forming genus *Diploschistes* Norman includes crustose species with a blackish pseudoparenchymatous proper exciple, lateral paraphyses, and a trebouxioid photobiont (Lumbsch 1989, Lumbsch & Mangold 2007, Lumbsch & Huhndorf 2010). The genus is widely distributed in arid and semiarid regions worldwide, with c. 43–45 species (Kirk & al. 2008, Pérez-Vargas & al. 2012, Fernández-Brime & al. 2013, Abbas & al. 2014, Ababaikeli & al. 2016). Most *Diploschistes* species occur on rocks, some on soil, and a few species are occasionally found on wood or bark (Lumbsch & Mangold 2007, Ababaikeli

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& al. 2016). The genus exhibits a remarkable range in the morphology of the ascomata, varying from perithecioid to urceolate or lecanoroid (Lumbsch 1989, Lumbsch & Mangold 2007). Despite this variability, molecular studies support the genus as monophyletic within the *Graphidaceae* (Frisch & al. 2006, Martín & al. 2003, Fernández-Brime & al. 2013). The few phylogenetic studies on *Diploschistes* published to date (Lumbsch & Tehler 1998, Martín & al. 2003, Fernández-Brime & al. 2013, Zhao & al. 2017) investigated the taxonomic value of ascoma morphology for delimiting monophyletic entities both at the generic and species levels.

The lichen biota of Northwestern China is rich, with more than 750 species belonging to about 150 genera listed so far (Abbas & Wu 1998, Abbas & al. 2001, Guo 2005). Although its lichen diversity remains incompletely investigated, continuing surveys should reveal more new species from this region. Five of the ten *Diploschistes* species recorded from China were described from Northwestern China (Wei 1991, Guo 2005, Abbas & al. 2014, Ababaikeli & al. 2016).

A new *Diploschistes* species, recently collected from Xinjiang, Northwestern China, is described here as *D. wui*. A taxonomic study of the new species is presented, based on the morphological and chemical characters. ITS nrDNA sequences were obtained from the holotype and two other specimens were compared with sequences available in GenBank to assess the phylogenetic affinities of the new species.

Materials & methods

Specimens were collected from Bayi forest farm in Changji county, Urumqi city, Xinjiang, China, and have been deposited in the Herbarium Mycologicum Academiae Sinicae-Lichenes, Beijing, China (HMAS-L) and the Lichen Section of Botanical Herbarium, Xinjiang University, Urumqi, China (XJU). The morphology of the lichen specimens was examined using a Zeiss Stemi SV 11 stereomicroscope. Sections for anatomical examination were cut by hand using a razor blade and were mounted and observed in water. Anatomical structure and hymenial characters were studied with a Zeiss Axioskop 2 plus light microscope. The photos were taken with a Nikon Digital Camera D50. Chemical constituents were identified by thin-layer chromatography using solvent system C as described by Orange & al. (2010).

DNA extraction, amplification, sequencing

Thallus fragments with ascomata were removed from the holotype and two other specimens for DNA extraction, using the DNAsecure Plant DNA Kit following the manufacturer's protocol. Amplification of the ITS region followed the methods described in Martín & al. (2003) modified as in Abbas & al. (2014). The nrITS region (ITS1+5.8S+ITS2) of the nrDNA repeat tandem was amplified with the polymerase

chain reaction using primers ITS1 with ITS4 (White & al. 1990) in a 25 µL volume containing 0.75 units of TransStart Taq Polymerase, 2.5 µL ITS buffer, 0.5 µl 5 µM primer solution, 2 µL 2.5 mM for each dNTP solution, and 1 µL genomic DNA. Thermocycling protocols followed were: 95°C for 5 min followed by 35 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min, and ending with a final extension of 72°C for 10 min. PCR products were screened on 1% agarose gels stained with ethidium bromide, and were sequenced by Genewiz Inc. (Suzhou, China).

All newly obtained sequences were submitted to GenBank. Similarities were determined from Blast results of sequence data, morphological characters, and the literature (Martín & al. 2003; Fernández-Brime & al. 2013, 2015; Zhao & al. 2017). Newly generated sequences from the Xingjiang material were complemented with GenBank sequences representing the most similar taxa.

Phylogenetic analysis

The ITS sequences from our three specimens and the 14 GenBank representatives were aligned both by ClustalW and Muscle implemented in MEGA7 (Kumar & al. 2016). The final matrix, submitted to TreeBase, can be obtained from the corresponding authors.

The evolutionary history was inferred by using the Minimum Evolution method implemented in MEGA7 (Kumar & al. 2016). The evolutionary distances were computed using the Maximum Composite Likelihood method; units represented number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 5). The Minimum Evolution (ME) tree was searched using the Close-Neighbor-Interchange (CNI) algorithm at a search level of 1. The initial tree was generated by the Neighbor-joining algorithm. The gaps were removed for each sequence pair. There were a total of 513 positions in the final dataset. The same dataset was also analyzed by Bayesian inference based on GTR model with rates = Invgamma.

Results & discussion

ITS sequences and the phylogenetic analysis

Almost the entire ITS region was successfully sequenced for the holotype and two additional specimens. Partial sequences (about 25 bp) were not obtained at the ITS1 5′ end, but partial sequences of the LSU 5′ end in region were included in the data submitted to GenBank (494, 495, and 480 bp in total).

The final nrITS alignment included 17 sequences representing 7 taxa and comprised 513 characters, of which 441 were constant, 18 were parsimony-uninformative, and 54 were parsimony-informative. There were no ambiguously aligned regions.

ITS sequence analysis places our specimens in either the *D. scruposus*-group (sensu Martín & al. 2003) or *D.* subg. *Diploschistes* (sensu Fernández-Brime

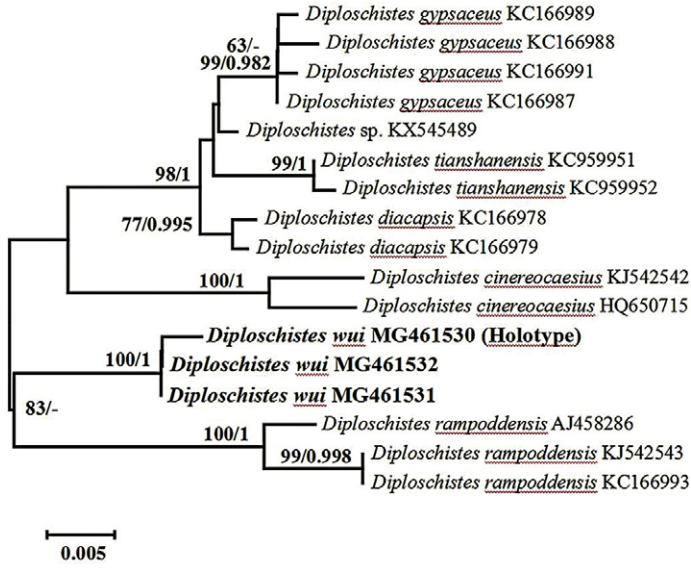

Fig. 1. An unrooted tree of 17 *Diploschistes* ITS sequences showing the phylogenetic relationships of *D. wui* and its closely related species. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap support ≥50% (BP; left) and posterior probabilities ≥0.95 (PP; right) are shown above branches. Newly generated sequences are presented in bold font. Internodes with both BP values ≥70% and PP ≥0.95 are highlighted by thicker lines.

& al. 2013) with close affinities to the pantropical species *D. rampoddensis* (93% identity and 2% gap). The evolutionary history was inferred as the minimum evolution tree (sum of branch length = 0.11303133). In the phylogenetic analysis, the relationship of the new species and its closely related species is clear (Fig.1).

Taxonomy

Diploschistes wui A. Abbas, S.Y. Guo & Ababaikeli, **sp. nov.** Fig. 2

FungalName FN 570518

Similar to *Diploschistes rampoddensis*, but distinguished by its thin, bluish gray thallus, its epruinose discs, its smaller 8-spored asci, and its smaller ascospores.

Type: China. Xinjiang: Urumqi, Changji County, Bayi forest farm, 43°22.82′N 86°48.22′E, alt. 1830 m, 3 Aug. 2009, A. Abbas 20093003 (**Holotype,** HMAS-L; GenBank MG461530; **Isotype,** XJU).

Etymology: The new species name honors Professor Jinong Wu, a pioneer Chinese lichenologist and lichenology advisor of author Abdulla Abbas.

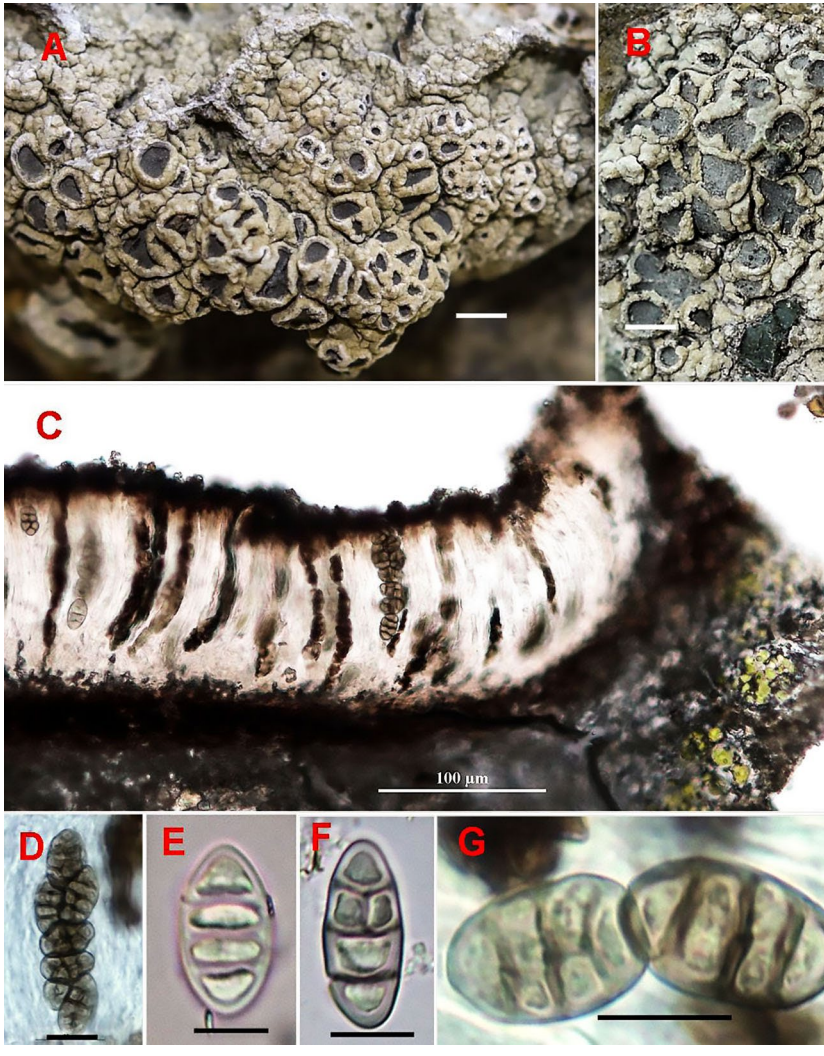

Fig. 2. *Diploschistes wui* (A, C–G = holotype, HMAS-L Abbas 20093003; B = XJU Abbas 20093004). A, B: general habit and ascomata; C: section through ascoma; D: ascus with ascospores; E–G: mature ascospores. Scale bars: A, B = 2 mm; C = 100 µm; D = 20 µm; E–G = 10 µm.

Thallus saxicolous, crustose, rimose-areolate, bluish grey to grayish white; areoles 0.3–0.6 mm diam., thin, usually <1.0 mm thick. Upper surface smooth, shiny or dull, epruinose. Medulla white. Photobiont trebouxioid with cells

≤10–13 µm diam. Prothallus not visible. Vegetative propagules absent. Ascomata apothecia, exposed. Disc urceolate, sessile, epruinose, blackish, concave, orbicular, 0.8–2.2 mm diam. Proper exciple dark brown, 60–80 µm thick. Hymenium hyaline, 80–100 µm high, not inspersed. Hypothecium yellowish brown, 20–40 µm thick. Paraphyses 1–2 µm thick, simple, apices not thickened. Asci cylindrical, 70–90 × 15–20 µm, 8-spored. Ascospores ellipsoid, brown, submuriform, with 3–5 transverse and 0–2 longitudinal septa, 10–15(–18) × 6–9 µm. Pycnidia unknown.

Spot tests—K–, C+ red, KC–, PD–, UV–.

Secondary metabolites—Only lecanoric acids detected by TLC.

Ecology—*Diploschistes wui* grows on rocks (mainly on flat surface of basic rocks). It is known only from the type locality in Xinjiang, Northwestern China, at elevation above 1800 m.

Additional specimens examined—**CHINA**. **Xinjiang**: **Urumqi,** Changji county, Bayi Forest farm, 43°22.82′N 86°48.22′E, alt. 1830 m, 15 July 2009, A. Abbas 20093004 (XJU; GenBank MG461531); 20093021(XJU; GenBank MG461532).

Comments—*Diploschistes wui* is very similar to the (6–)8-spored specimens of *D. rampoddensis* (Nyl.) Zahlbr., in both morphology and chemistry. However, *D. rampoddensis* differs in its grayish yellow to greenish yellow thallus with a crystalliferous surface, pruinose discs, and a usually pantropical distribution (Lumbsch 1993; Pant & Upreti 1993; Fernández-Brime & al. 2013, 2015).

The pantropical species *Diploschistes cinereocaesius* (Sw.) Vain., which resembles *D. wui* in its octosporous asci and somewhat similar ascospore size, is distinguished by its terricolous habit, larger apothecia that are usually secondarily subdivided, and a different chemistry, containing both lecanoric acid (major) and diploschistesic acid (minor) (Fernández-Brime & al. 2015). Another morphologically similar species with an epruinose thallus is *D. scruposus* (Schreb.) Norman., which differs in its (4–)8-spored asci and bigger ascospores (25–40 × 10–20 µm) (Ababaikeli & al. 2016).

A recent multilocus phylogeny of selected *Diploschistes* species demonstrated that only a few species in *D.* subg. *Diploschistes* are strongly supported as monophyletic, while *D. diacapsis*, *D. interpediens*, *D. muscorum*, *D. neutrophilus*, and *D. scruposus* are not monophyletic (Zhao & al. 2017). We feel that monophyly is a prerequisite for species recognition in single-locus ITS phylogenies. Other studies using DNA sequence data for species recognition in lichens support our opinion (e.g. Han & al. 2013, 2015; Abbas & al. 2014; Ababaikeli & al. 2016). Our morphological and phylogenetic analyses support recognition of *Diploschistes wui* as an independent species.

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